DETERMINATION OF RESIDUES OF EIGHT SYNTHETIC PYRETHROIDS IN TOBACCO BY CAPILLARY GAS CHROMATOGRAPHY

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SUMMARY

A method was developed for the simultaneous determination of the residues of the following eight synthetic pyrethroids and their isomers in tobacco: Tetramethrin, Permethrin, Cyfluthrin, Cypermethrin, Alfamethrin, Flucythrinate, Fluvalinate and Deltamethrin. The pesticides were extracted from ground tobacco with acetone:water 9:1 for 5 hours. The extract was diluted with water and partitioned into n-hexane. The organic phase was concentrated to about 1 ml and then purified by a Florisil-SPE column. The elution was carried out according to the following pattern:
1) fraction 1 = hexane (3 ml)
2) fractions from 2 to 8 = hexane:diethylether 9:1 (2 ml each)
3) fraction 9 = diethylether (20 ml).
Tetramethrin was found in fraction 9. The other pyrethroids in the fractions from 4 to 8.
The gas-chromatographic analyses were run with a gas-chromatograph Carlo Erba Series Mega HRGC 5300 equipped with a capillary column (stationary phase OV-1 - 0.10-0.15 µm film thickness, 25 m long) and a ^{63}Ni electron capture detector. Two different injection ports were used: split-splitless and cold split-splitless, working both with isothermal and programmed temperatures. The latter shows chromatograms with lower drift and lower noise, while the former gives a higher sensitivity. Both the limit of detection and the limit of determination were estimated for each compound. Recoveries from fortified samples at level of 1 ppm are reported.

INTRODUCTION

The pyrethroid insecticides are the object of intense interest for use in tobacco protection because of their desirable environmental properties of short persistence and nontoxicity to mammals. These features, combined with their broad spectrum of insecticidal activity and with their toxicological properties which permit the control of certain insects at very low application rates, have made the pyrethroids environmentally safe and acceptable alternatives to the older organochlorine compounds and to the natural pyrethrins. The rapid increase in their application and the recent commercial introduction of some new pyrethroids compounds require a method by which the residues of as many pyretrhroids as possible can be determined.

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Single-residue and multiresidue methods for the determination of pyrethroid insecticides have been reported (1-8). These compounds are easily analyzed by gas-liquid chromatography because of their thermal stability and electron capturing properties. However the non specific nature of ECD introduces the further requirement that a methodology be developed to provide interference-free extracts.

To this end in the present method Solid Phase Extraction was used for clean-up step. Solid Phase Extraction (SPE) is a rapid and efficient sample preparation technique based on separation mechanisms of liquid chromatography. SPE tubes are prepared by suppliers in the same manner as corresponding packings in HPLC columns. This packings ensure highly reproducible extractions and a sample recovery usually greater than 95% and often very close to 100%.

The present method was tested for simultaneous determination of residues in tobacco of the following eight synthetic pyrethroids and their isomers: Tetramethrin, Permethrin, Cyfluthrin, Cypermethrin, Alfamethrin, Flucythrinate, Fluvalinate and Deltamethrin.

EXPERIMENTAL

-Apparatus and Reagents.

A gas-chromatograph Carlo Erba Series Mega mod.5300 equipped with a split-splitless or a cold split-splitless injector, a temperature programmer and a ^{63}Ni electron-capture detector(ECD) was used. The ECD was operated at a temperature of 300 °C in the constant period mode with a pulse width of 1 μsec. The chromatographic column was a 25 m x 0.32 mm i.d. capillary column with an OV-1 stationary phase of film thickness 0.10-0.15 μm. The carrier gas was helium with a flow rate of 0.5 ml/min. Additional make-up gas (nitrogen) was added to the capillary effluent at a flow rate of 50 ml/min.

The gas-chromatographic conditions were the following:

a) Split-splitless injection system

Injector temperature = 250 °C

Oven temparature = isothermal at 215 °C for 18 min followed by temperature programming to 220 °C at 5 °C/min, isothermal at 220 °C for 18 min and temperature programming to 250 °C at 10 °C/min. The final temperature was mantained for 8 min.

The split ratio was 1:10.

b) Cold split-splitless system

Injector temperature = initial 60 °C, final 250 °C

Splitless time = 10 sec. Purge time = 1 sec.

The other chromatographic conditions were as above.

In all cases the chromatograms were recorded by using a HP3699 computing recorder.

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Supelclean disposable SPE tubes (LC-Florisil 6 ml, 1 g) filled with Florisil were used.
All the reagents were pesticide-grade.
Pyrethroids stock standard solutions (1000 µg/ml in hexane) are stable for at least 1 month if stored in the dark at 4 °C. Working standard solutions were prepared daily.
As internal standard mirex can be used at a concentration of 5 µg/ml.

-Procedure.
Weigh 2 grams of ground tobacco in a jar, add 100 ml of acetone:water 9:1 and extract for 5 hours using a mechanical stirrer. After filtration through filter paper Whatman N.3 or equivalent, transfer the solution into a separatory funnel and extract with 4 x 25 ml portions of hexane. Filter the combined portions of organic phase through anhydrous sodium sulphate and evaporate to about 1 ml with vacuum rotatory evaporator at 40 °C. Using a Pasteur pipette transfer the hexane extract onto a short column of anhydrous sodium sulphate on the top of a SPE tube previously conditioned with 2 ml of hexane. Elute with hexane (3 ml) followed by seven fractions (2 ml each) of a mixture hexane:diethylether 9:1 and then by diethylether (20 ml). Discard the first three fractions and collect the other ones. In table 1 the pyrethroids distribution in the fractions eluted from SPE tube is shown. Combine the collected fractions, concentrate the resulting solution to 2 ml by using a stream of clean, dry nitrogen and inject 1 µl into gas-chromatograph. It is possible, of course, to inject into gas-chromatograph 1 µl of each fraction eluted from SPE tube.

TABLE 1
SYNTHETIC PYRETHROIDS DISTRIBUTION IN SPE-FRACTIONS

Pyrethroids	SPE-Fractions					
	4	5	6	7	8	9
Permethrin	++	--	--	--	--	--
Cyfluthrin	++	++	++	--	--	--
Cypermethrin	++	++	++	--	--	--
Alfamethrin	++	++	++	--	--	--
Deltamethrin	--	--	++	++	--	--
Fluvalinate	--	--	++	++	--	--
Flucythrinate	--	--	--	++	++	--
Tetramethrin	--	--	--	--	--	++

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RESULTS AND DISCUSSION

Figures 1 and 2 show typical chromatograms obtained by injecting 1 µl of mixed standard pyrethroids solution (1 µg/ml each) into gas-chromatograph equipped respectively with split-splitless and cold split-splitless injection port. The numbers of the peaks in both figures correspond to the compounds listed in table 2. It is clear that the latter system shows chromatograms with lower drift and lower noise, while the former gives a higher sensitivity for some compounds.

TABLE 2
GAS-CHROMATOGRAPHIC PEAKS OF SYNTHETIC PYRETHROIDS

Pyrethroids	Peak Numbers
Tetramethrin	1-2
Permethrin	3-4
Cyfluthrin	5-6-7-8
Cypermethrin	9-10-11-12
Alfamethrin	11
Flucythrinate	12-13
Fluvalinate	14-15
Deltamethrin	16

While the stereo-isomers of each pyrethroid are resolved by the capillary column used, it was unable to separete a Cypermethrin isomer from one of Flucythrinate. However these compounds can be easily determined as they are present in different fractions of SPE tube eluate.

As shown in figure 3, no coextractant from untreated tobacco interferes with the pyrethroids tested.

All synthetic pyrethroids examined were quantitatively analyzed under GLC conditions given above by determining the sum of their isomers. Detector responses were linear in the range 0.01-1 µg/ml. For each compound the calibration curve, based on peak area measurement, was a straight line and passed through the origin. The correlation coefficients were in the range 0.9989-0.9999.

Table 3 shows the limits of detection and the limits of determination, estimated for each pyrethroid according to OFC Pesticide Commission Method (9).

The recovery at 1 ppm level was checked by adding known volumes of a mixed pyrethroid standard solution in hexane to five suitable portions of tobacco shown not to contain residues of the pyrethroids. The samples were then treated as described in "Procedure". The results obtained are reported in table 4.

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TABLE 3

DETECTION AND DETERMINATION LIMITS FOR SYNTHETIC PYRETHROIDS

Pyrethroids	Detection limit (ppm)	Determination limit (ppm)
Alfamethrin	0.01	0.02
Cyfluthrin	0.02	0.05
Cypermethrin	0.01	0.02
Deltamethrin	0.01	0.02
Flucythrinate	0.02	0.05
Fluvalinate	0.01	0.02
Permethrin	0.01	0.02
Tetramethrin	0.01	0.02

TABLE 4

RECOVERY OF SYNTHETIC PYRETHROIDS FROM FORTIFIED TOBACCO

Pyrethroids	Recovery %
Alfamethrin	95
Cyfluthrin	97
Cypermethrin	95
Deltamethrin	110
Flucythrinate	108
Fluvalinate	104
Permethrin	95
Tetramethrin	90

Finally, as an example of routine determination, in figures 4-6 some chromatograms of field-treated tobacco samples are shown.

In conclusion, an effective procedure has been developed for the extraction of eight synthetic pyrethroids from tobacco and for a simple and rapid clean-up of these extracts in order to carry out the analysis of the pyrethroids at the residue level by GLC-ECD.

On the basis of structural similarities, one can assume that this method, which has been successfully used in our laboratory for routine determinations, can be extended for the residues analysis of other pyrethroid insecticides.

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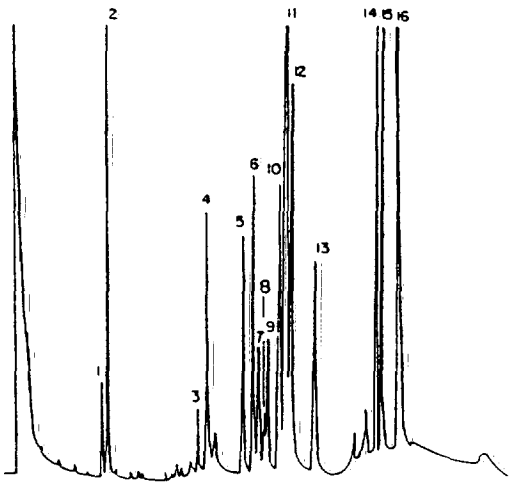

Fig. 1 - GASCHROMATOGRAM OF A STANDARD MIXTURE OF SYNTHETIC PYRETHROIDS OBTAINED BY USING SPLIT-SPLITLESS INJECTION PORT. THE NUMBERS OF THE PEAKS CORRESPOND TO THE COMPOUNDS REPORTED IN TABLE 2.

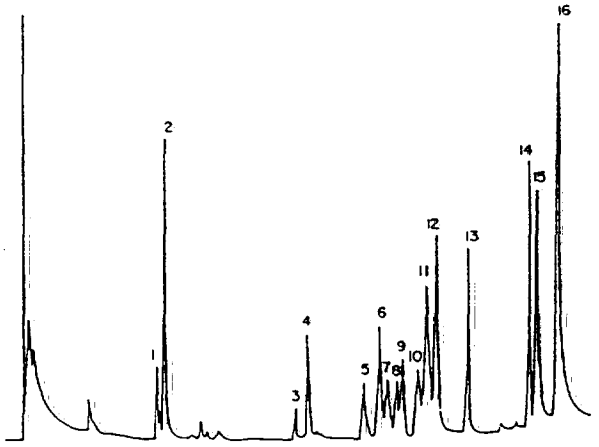

Fig. 2 - GASCHROMATOGRAM OF A STANDARD MIXTURE OF SYNTHETIC PYRETHROIDS OBTAINED BY USING COLD SPLIT-SPLITLESS INJECTION PORT. THE NUMBERS OF THE PEAKS CORRESPOND TO THE COMPOUNDS REPORTED IN TABLE 2.

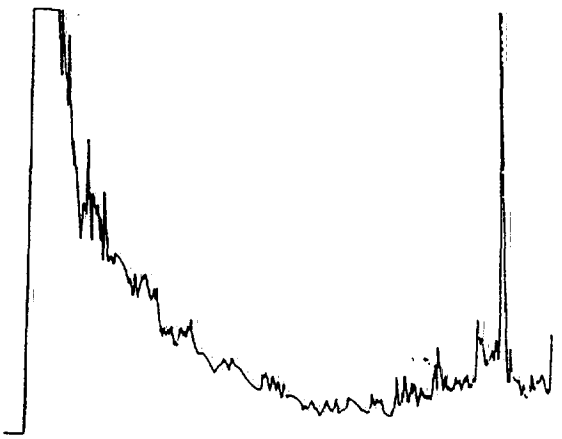

Fig. 3 - GASCHROMATOGRAM OF UNTREATED TOBACCO

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